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Changes after Decapitation in Concentrations of Indole-3-Acetic Acid and Abscisic Acid in the Larger Axillary Bud of *Phaseolus vulgaris* L. cv Tender Green[1]

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ABSTRACT

Early changes in the concentrations of indole-3-acetic acid (IAA) and abscisic acid (ABA) were investigated in the larger axillary bud of 2-week-old *Phaseolus vulgaris* L. cv Tender Green seedlings after removal of the dominant apical bud. Concentrations of these two hormones were measured at 4, 6, 8, 12 and 24 hours following decapitation of the apical bud and its subtending shoot. Quantitations were accomplished using either gas chromatography-mass spectrometry-selected ion monitoring (GC-MS-SIM) with [$^{13}C_6$]-IAA or [$^{2}H_6$]-ABA as quantitative internal standards, or by an indirect enzyme-linked immunosorbent assay, validated by GC-MS-SIM. Within 4 hours after decapitation the IAA concentration in the axillary bud had increased fivefold, remaining relatively constant thereafter. The concentration of ABA in axillary buds of decapitated plants was 30 to 70% lower than for buds of intact plants from 4 to 24 hours following decapitation. Fresh weight of buds on decapitated plants had increased by 8 hours after decapitation and this increase was even more prominent by 24 hours. Anatomical assessment of the larger axillary buds at 0, 8, and 24 hours following decapitation showed that most of the growth was due to cell expansion, especially in the internodal region. Thus, IAA concentration in the axillary bud increases appreciably within a very few hours of decapitation. Coincidental with the rise in IAA concentration is a modest, but significant reduction in ABA concentration in these axillary buds after decapitation.

Thimann and Skoog (17) were the first to postulate an inhibitory role for the auxin, IAA, with respect to apical dominance. The first definitive measurements of IAA in an apical dominance system were made by Hillman et al. (9) with *Phaseolus vulgaris* L. cv Canadian Wonder and indicated that the concentration of IAA in the two (grouped) axillary buds had doubled by 24 h following decapitation. By this time the larger axillary bud of his decapitated plants had extended, on average, 1.9 mm more than the equivalent bud on intact plants.

We have also investigated the phenomenon of apical dominance in *P. vulgaris* L. plants by attempting to characterize the timing of changes in endogenous IAA and ABA concentrations in the larger axillary bud for intact and decapitated plants. The larger axillary bud was selected for analysis since it invariably becomes the dominant bud following decapitation. We included ABA in our analyses since it is a potential antagonist of other growth hormones, including IAA. Thus, we have also examined the hypothesis (8) that ABA concentrations decrease during the release of buds from correlative inhibition.

MATERIALS AND METHODS

Plant Materials

In each set of experiments *Phaseolus vulgaris* L. cv Tender Green were grown in 30 × 60 × 10-cm plastic trays, filled with a 1:1 mixture of sand and peat moss, under a 16-h photoperiod at 23°C and 60% RH in a growth chamber (Controlled Environment Systems model PGV 36LT Winnipeg, Man.). Lighting was provided by high-pressure mercury lamps 250 μE $m^{-2}s^{-1}$ PPFD. Fourteen days after planting, uniform seedlings were selected on the basis of height, leaf size, and size of both apical and axillary buds. These plants were randomly divided into groups of 60 plants (3 trays of 20 plants). In one-half of the plants the apical bud was removed by decapitation and in the other half the plants were left intact. Trays containing decapitated or intact plants were randomized in the chamber. At 2, 4, 6, 8, 12, and 24 h after decapitation the larger axillary bud was excised from each of the 60 plants for each of control and decapitated groups, and immediately immersed in liquid N_2. All frozen tissues were lyophilized. Duplicate samples (each from 60 plants) of tissue were taken at each harvest for IAA and ABA analysis by GC-MS-SIM.[2] In a second complete experiment axillary buds from another group of plants were harvested solely for analysis of ABA by ELISA. The harvest times for examining ABA by ELISA were every 0.25 h up to 2 h, then every 0.5 h to 12 h, and hourly thereafter. The number of buds harvested for each ELISA was six, for each of decapitated and control plants.

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[2] Abbreviations: GC-MS-SIM, gas chromatography-mass spectrometry-selected ion monitoring; BHT, butylated hydroxytoluene; DCMS, dichlorodimethylsilane; d-t-BDMS-IAA, ditertbutyldimethylsilyl-IAA; HOAc, acetic acid; MeOH, methanol; MSD, mass selective detector; MTBSTFA, *N*-[*tert*-butyldimethylsilyl]-*N*-methyltrifluoroacetamide; Rt, retention time.

Extraction

IAA and ABA were processed separately because of different pH requirements for safe extraction. Aliquots (triplicate, in serial dilutions) of the 80% aqueous MeOH extract were immediately taken for analysis of ABA by ELISA. The residual MeOH extract then had [$^2H_6$]-ABA added as a quantitative internal standard. ELISA, validated by GC-MS-SIM, became the preferred method of analysis for ABA since large numbers of tissue samples could be processed with minimal purification.

Lyophilized tissue was extracted for IAA in 80% aqueous MeOH with 100 mg of the sodium salt of L-ascorbic acid (Sigma) per 200 mL of pH 6.5 H_2O and 200 mg of BHT (Sigma) in 800 mL of MeOH (7). For extracts of IAA to be analyzed by GC-MS-SIM all glassware was silylated with DCMS. Lyophilized axillary buds from 60 plants (10–20 mg dry weight) were ground to a fine powder in a small glass homogenizer and extracted using 5 mL of 80% aqueous MeOH at 3 to 5°C. This solution was then filtered and the tissue residue and filter paper (Whatman No. 1) reextracted with another 5 mL of 80% aqueous MeOH another two times. The pooled extracts (15 mL) were then diluted to 20 mL with additional 80% aqueous MeOH and adjusted to pH 6.5. At this point 100 ng of [$^{13}C_6$]-IAA (Merck) was added as a quantitative internal standard (5) as was 333 Bq of [^{3}H]-IAA (619 GBq/mmol, Amersham). The latter allowed for the ready determination of the IAA Rt by HPLC. ABA extractions also utilized 80% aqueous MeOH with antioxidants. However, the pH was maintained at 3.0. Extracts for ABA analysis by GC-MS-SIM had 100 ng of [$^2H_6$]-ABA added as a quantitative internal standard along with 333 Bq of [^{3}H]-ABA (374 GBq/mmol, Amersham) for ready determination of HPLC Rt. These internal standards were added immediately after an aliquot was removed for use in the ABA ELISA.

Purification of IAA Samples

The IAA MeOH extract was passed through a syringe barrel column (3 cm i.d. × 11 cm) packed with 2.0 g of preparative grade C_{18} matrix (55–105 μm particle size, PrepPak 500/C_{18}, Waters Scientific). This column was termed 'C_{18}-PC.' It was first conditioned with 2 × 25 mL of 100% MeOH, then equilibrated by elution with 3 × 25 mL of pH 6.5 80% aqueous MeOH at a flow rate of one drop/s. The MeOH extract for IAA analysis was then passed through the C_{18}-PC and the eluate was diluted to 30 mL with an 80% aqueous MeOH rinse. Duplicate aliquots of 1 mL were taken from the total eluate for radioassay.

The C_{18}-PC eluate was then taken to near dryness *in vacuo* on a rotary flash evaporator at 30°C and diluted to 5 mL with pH 2.8 H_3PO_4 buffer. This solution was immediately passed through a C_{18} Sep Pak cartridge (Waters Associates) that was prewashed and eluted with newly made water-saturated BHT-diethyl ether. The ether fraction, which contained IAA, was evaporated in a fumehood by a gentle flow of N_2 with hand warming.

The dried eluates of the ether-soluble fraction were dissolved in 24.4% MeOH in 1% aqueous HOAc (initial conditions) and chromatographed on a Waters Associates (Mississauga, Ontario) reversed phase C_{18} μ-Bondapak column (3.9 mm i.d. × 300 mm). The HPLC was as per Koshioka *et al.* (11). The fractionation program used was 0 to 30 min isocratic gradient of 24.4% MeOH, 30 to 40 min linear gradient from 24.4 to 73.3% MeOH, 40 to 45 min linear gradient from 73.3 to 100% MeOH, followed by a 15 min 100% MeOH wash. The flow rate during the entire elution program was 2 mL/min. The column was equilibrated for 20 min at initial conditions before injection of each sample. The Rt of [^{3}H]-IAA was determined by liquid scintillation spectrometry of a one-tenth aliquot taken from each 1 min HPLC fraction. The approximate Rt of IAA on this column was 15 min.

All radioactive HPLC fractions were pooled, taken to near dryness *in vacuo* with a rotary flash evaporator, and dissolved a small volume (1.2 mL) of 10% isopropanol containing 0.2 M imidazole (Kodak, recrystallized from MeOH) buffer at pH 7.0. The isopropanol solution was then applied to a conditioned 0.3 g PrepSep NH_2 amino anion exchange column (Fisher) (4). IAA was eluted by acidic MeOH and this eluate was dried quickly under a gentle flow of N_2 with hand warming and stored unless the extract showed visual color. If so, it was then dissolved in 4 × 300 μL of 99.9% MeOH with 0.1% HOAc and loaded on a 4.6 mm i.d. × 150 mm $N(CH_3)_2$ Nucleosil HPLC column packed with 5 μm particles (19) (Alltech Associates Inc., Deerfield, IL). The column was eluted with 99.9% MeOH with 0.1% HOAc, at a flow rate of 1 mL/min. The fractions containing [^{3}H]-IAA were pooled and dried quickly under a gentle flow of N_2 with hand warming. The Rt of [^{3}H]-IAA on the Nucleosil column was about 6 min.

GC-MS-SIM Analysis of IAA

The IAA-containing fraction from HPLC was then derivatized (6). In essence, the dry residue was dissolved in 40 μL of acetonitrile and 20 μL of MTBSTFA (Pierce, Rockford, IL) was added. The vial was flushed with N_2 and tightly capped, and the mixture was heated for 15 min at 70°C. The sample was dried with N_2 and immediately analyzed by GC-MS-SIM using dry *n*-hexane as the injection solvent. The ions m/z 409/403 to 352/346 and 250/246 were monitored and their relative intensities used to identify [$^{13}C_6$]-IAA and IAA. Endogenous IAA concentrations were quantified from a standard curve that established a ratio of peak areas of the ions (m/z 409/403) over a range of precisely known quantities of both [$^{13}C_6$]-IAA and IAA.

We used a Hewlett-Packard 5790A GC with a capillary column directly interfaced to a model 5970b MSD. The GC temperature was programmed from 60 to 165°C at 25°C/min, immediately followed by a 5°C/min ramp to 275°C. The GC column was a 15.0 m DB1 fused silica capillary column (J and W Scientific; 0.25 mm i.d., 0.25 μm film thickness). The Rt for di-t-BDMS-IAA was 19.62 ± 0.05 min.

Purification of ABA Samples

The portion of the extract (usually 90%) to be quantified by GC-MS-SIM was passed through a C_{18}-PC (as above for IAA), except that 80% MeOH at pH 3 was used as the eluting solvent.

After removal of the MeOH *in vacuo*, the eluate was subsequently frozen then lyophilized, and the residue was dissolved in four rinses of 300 μL of 24.4% MeOH in 1% HOAc. Using the same C_{18} HPLC gradient program described above, the entire sample was injected into the HPLC and a 10% aliquot of each fraction was taken for locating [^{3}H]-ABA by liquid scintillation spectrometry. The Rt for ABA was about 39 min using this program. The major radioactive fractions were pooled and dried *in vacuo*. A second HPLC run was always performed using the Nucleosil $N(CH_3)_2$ column (Alltech Associates Inc.) as described above. ABA eluted from $N(CH_3)_2$ HPLC at about 8 min and the [^{3}H]-ABA-containing fractions were grouped and dried *in vacuo*.

GC-MS-SIM Analysis of ABA

The residue was derivatized, as described above, with MTBSTFA in acetonitrile for 15 min at 70°C, then dried. Only the mono-t-BDMS derivative of ABA was obtained (6). The dried sample was then injected onto GC-MS-SIM in dry *n*-hexane using the same temperature program as described for IAA. Ions m/z 327/321 to 241/237 and 194/190 were monitored and the relative intensities used to identify [$^2H_6$]-ABA/ABA. As described for IAA, a calibration curve for ABA was created using the area ratios of the intense peaks (m/z 321 for ABA and m/z 327 for [$^2H_6$]-ABA) plotted against ratios of known amounts of each of ABA and [$^2H_6$]-ABA.

ABA ELISA

Using the indirect method of Ross *et al.* (14), 10% aliquots from certain ABA MeOH extracts were analyzed by ELISA using monoclonal antibody purchased from Idetek (1057 Sneath Lane, San Bruno, CA). Before ELISA, a one step pH 3, 50% aqueous MeOH C_{18}-PC procedure was used to yield a paritially purified extract (14) containing the majority of the [^{3}H]-ABA internal standard. This eluate was dried under reduced pressure. Serial dilutions (200^{-1}, 400^{-1}, 800^{-1}, 1600^{-1}, and 3200^{-1}) in triplicate were then made with MeOH and taken to dryness prior to ELISA. Immediately before ELISA the serially diluted aliquots were dissolved in a very small volume (10 μL) of MeOH and diluted to 200 μL with assay buffer. Freshly made standard ABA solutions of 1000, 500, 300, 200, 100, 80, 60, 30, 15, and 7.5 pg/50 μL were analyzed by ELISA in a similar ratio of MeOH:assay buffer (10:190). Quantities of ABA in the axillary buds of the four control extracts and four decapitated extracts for each of 2, 4, 8, and 24 harvests were analyzed by both ELISA and GC-MS-SIM. In seven out of eight comparisons (see "Results"), ABA concentrations were equivalent, and we considered this to be a reasonable validation of the ELISA. Given the reasonable correspondence of the ELISA results to GC-MS-SIM results, 54 separate extracts were made using six buds per extract, and each of these was analyzed by ELISA after the C_{18}-PC purification step so as to provide a more complete picture of ABA changes over time.

Anatomical Analysis

Axillary buds were excised and fixed in FAA (formalin-acetic acid-alcohol), dehydrated in tertiary-butyl-alcohol, and embedded in paraffin according to the general method of Jensen (10). Serial longitudinal sections (7 μm) were cut with an American Optical rotary microtome. Sections were stained in a mixture of basic fuchsin, safranin, and crystal violet (0.5%, 0.2%, and 0.2% in 50% ethanol, w:v) and counterstained with fast green (20).

RESULTS

The dry weight concentration of extractable IAA in axillary buds increased significantly over controls ($P \leq 0.05$) by 4 h after decapitation. The IAA concentrations in the larger axillary buds of decapitated plants showed no difference at 2 h from buds of intact plants, but by 4 h the IAA concentrations were 5 times higher than in the intact controls (Fig. 1). IAA attained a maximal concentration in the larger axillary bud by 8 h following decapitation. In the larger axillary buds of intact, control plants the IAA concentration remained relatively constant (Fig. 1).

The ABA concentrations were significantly lower ($P \leq 0.05$) in axillary buds of decapitated plants than in the intact control plants from 4 to 24 h following decapitation (Fig. 2; Table I). The ABA concentration at 24 h in axillary buds of intact plants was 2 to 4 times higher than in axillary buds from the decapitated plants. The ABA concentrations which were determined by GC-MS-SIM from samples of 60 buds (Fig. 2) were in good agreement with values obtained by ELISA for the same samples (Table I). The frequent sampling times used in the more extensive ABA ELISA (Fig. 3) also confirm that the ABA concentration in the axillary buds of decapitated plants was generally lower ($P \leq 0.02$) than in comparable buds from intact, control plants (Fig. 3).

A periodic wave in ABA concentration was apparent (Fig. 3), with the period of ABA concentration in buds from intact plants being shorter than in decapitated buds. The amplitude of the wave for ABA concentration in buds from intact plants

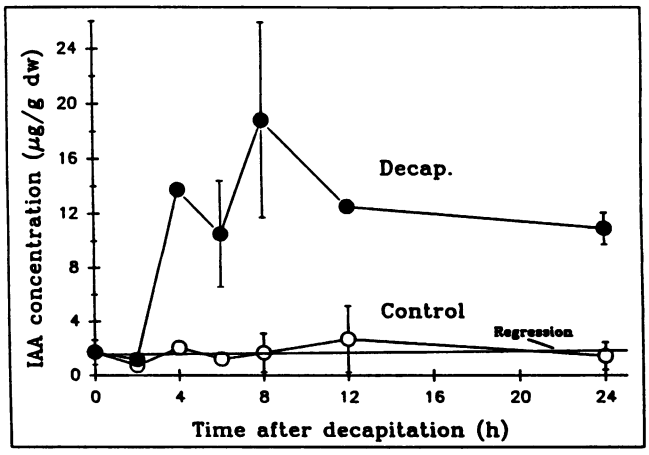

Figure 1. Effect of terminal shoot apex decapitation on the average concentration (dry weight basis) of endogenous IAA in the larger axillary buds of *P. vulgaris* L. cv Tender Green using GC-MS-SIM with [$^{13}C_6$]-IAA as a quantitative internal standard. Data are the mean of two replicate experiments, each using 60 buds (mean concentration ± SE [99% confidence limit]). (●), Decapitated; (○), intact.

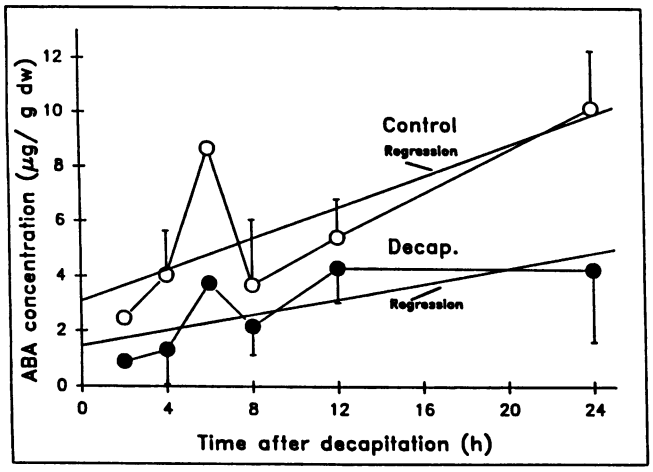

Figure 2. Effect of terminal shoot apex decapitation on the average concentration (dry weight basis) of endogenous ABA determined in the larger axillary buds of *P. vulgaris* L. cv Tender Green using GC-MS-SIM with [$^2H_6$]-ABA as a quantitative internal standard. Each point represents a single extraction using 60 buds. Regression analysis was performed with respect to each data point (2). (●), Decapitated; (○), intact.

Table I. *Effect of Terminal Shoot Apex Decapitation on the ABA concentration (dry wt basis) in the Largest Axillary Bud from 2-week-old Seedlings of P. vulgaris L. cv Tender green*

Data are given for two replicate controlled environment chamber experiments (I and II). ABA was quantified by ELISA using [^{3}H]-ABA as a quantitative internal standard for work-up losses up to the time of the ELISA.

Treatment	ABA Concentration (99% confidence limit)	
	Experiment I[a]	Experiment II[a,b]
	μg/g dry wt ± SE[c]	
Hour 2 control	7.40 ± 0.95	2.50 ± 0.37
Hour 2 decapitation	4.50 ± 1.22	4.82 ± 0.48
Hour 4 control	5.45 ± 0.39	6.07 ± 1.52
Hour 4 decapitation	3.63 ± 0.16	1.95 ± 0.34
Hour 8 control	3.18 ± 0.36	4.44 ± 0.90
Hour 8 decapitation	—[d]	1.36 ± 0.38
Hour 24 control	1.05 ± 0.16	13.37 ± 0.92
Hour 24 decapitation	0.61 ± 0.06	2.66 ± 0.63

[a] Each sample consisted of approximately 60 buds. [b] These data points are also shown, using GC-MS-SIM with [$^3H_6$]-ABA as a quantitative internal standard, for the residual portion of the 60 bud extract in Figure 2. Results of the two methods (ELISA and GC-MS-SIM) were analyzed using the paired *t* test (2), and method of analysis was a significant variable only at $P \leq 0.633$. [c] 99% confidence limit. [d] Sample lost during purification.

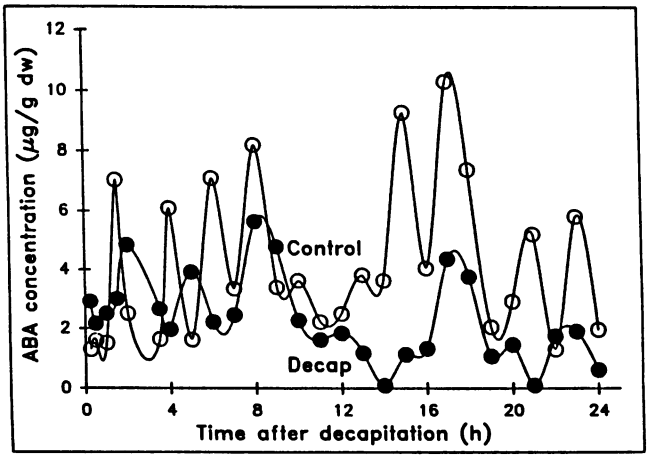

Figure 3. Effect of shoot apex decapitation on the ABA concentration and its diurnal fluctuations in the larger axillary buds of *P. vulgaris* L. cv Tender Green, as determined by ELISA. Each point represents one sample of six buds and is the average of three serial dilutions performed in triplicate and measured by ELISA (mean concentration ABA ± SE [99% confidence limit]). (●), Decapitated; (○), intact. The trend of lower ABA concentrations in axillary buds from decapitated plants is significant at $P \leq 0.05$ based on analysis by the paired *t* test (2). Regression analysis was performed with respect to each data point (2).

was also larger than in buds from decapitated plants. The amplitude and period for ABA concentration in buds of intact plants were, respectively, 4.9 μg/g dry weight and 2 h, whereas in buds of decapitated plants they were 2.2 μg/g dry weight and 3 h.

Axillary buds increased rapidly in size after decapitation of the terminal apex and subtending shoot (compare A and B of Fig. 4). All parts of the axillary bud from decapitated plants, *i.e.* the scale leaves, the major leaf flanking the shoot bud, and the terminal axillary shoot bud *per se*, were larger than these same parts from axillary buds of control, intact plants (Table II). Accompanying this was a fresh weight increase (relative to the controls), first measurable 8 h after decapitation (Fig. 5). The fresh weight increase was even more prominent at 24 h. During the same sampling times the tissue dry weight also increased. However, this increase was much smaller than that for fresh weight (Table II).

Anatomical sections revealed that the primary cause of bud enlargement was increased cell size. Cell enlargement was especially prominent in the internodal region of the axillary shoot bud. The pith parenchyma cells expanded radially, causing the axis to widen (compare A and B of Fig. 6). This was followed by repeated transverse cell divisions within the internode. The addition of new cells to the developing internode at this time probably account for the future rapid growth and elongation of the released axillary bud. Decapitation of the terminal apex also induced cell enlargement within the scale leaves of the larger axillary bud. Therefore, cell enlargement and the addition of more cells to the stem of the developing axillary bud/new shoot account for the overall size increase that occurs shortly after decapitation.

Both control and decapitated buds were absent of functional conducting tissue up to 24 h following decapitation.

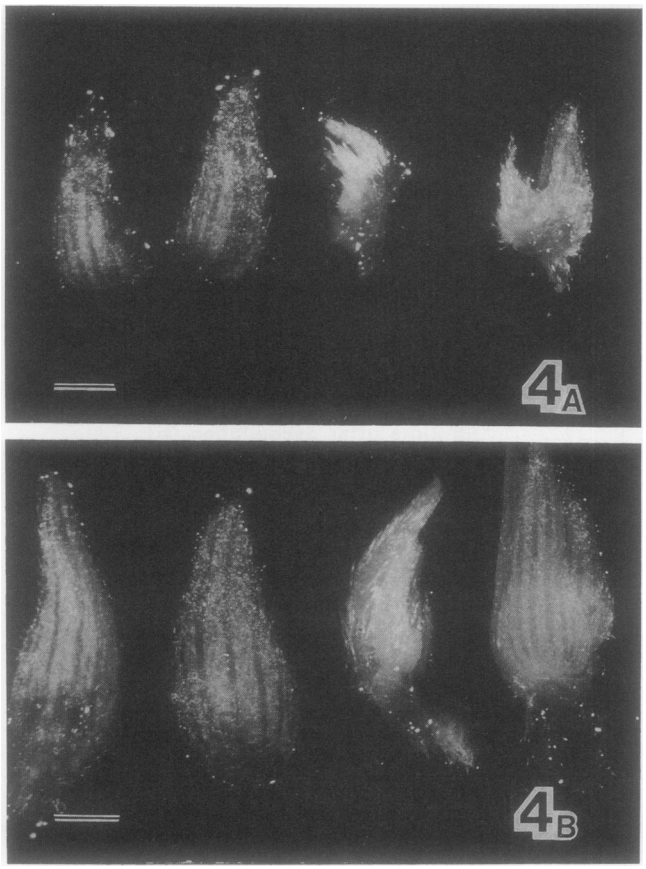

Figure 4. A, Photograph showing the major anatomical components of an axillary bud from a control plant sampled 24 h after removal (decapitation) of the shoot apex. Two scale leaves, a developing leaf flanking the shoot tip, and the shoot tip are arranged from left to right. B, Photograph showing the major components of an axillary bud sampled 24 h after removal (decapitation) of the shoot apex. All anatomical components of the axillary bud have increased in size. Scale bars = 1 mm.

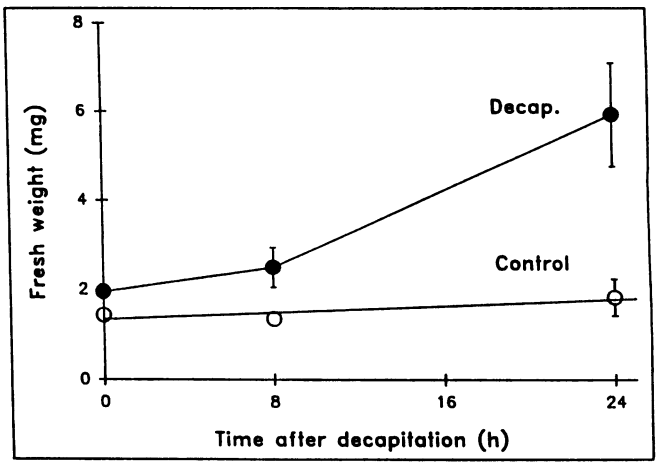

Figure 5. Effect of apical shoot decapitation on the increase in fresh weight of the larger axillary bud (mg ± SE [99% confidence limit]). (●), Decapitated; (○), intact.

DISCUSSION

Within 4 h after decapitation of the apical shoot the concentration of IAA increased fivefold (significant at $P \leq 0.007$), while the concentration of ABA decreased (significant at $P \leq 0.02$). A decreased ABA concentration was apparent by 2 and 4 h (Fig. 2), and peak concentrations (top of cycle period) of ABA were significantly ($P \leq 0.05$) lower in buds from decapitated plants from h 3 to 24 (Fig. 3). Due to relatively good sensitivity of the GC-MS-SIM technique (10 pg IAA or ABA), large numbers of buds were not necessary to quantitate either hormone. This contrasts with earlier work (8), where 4,000 to 10,000 axillary buds were needed. Reliable (accurate and precise) results for IAA and ABA were obtained by GC-MS-SIM with as few as 60 buds in the present work, providing that an appropriate purification method was used. However, rigorous selection of plants for axillary bud uniformity was crucial.

Table II. *Effect of Decapitation of the Terminal Shoot Apex of 2-week-old Seedlings of P. vulgaris L. cv Tender Green on Fresh Weight (mg ± SE [99% confidence limit]) of the Larger Axillary Lateral Bud*

Time	Portion of Bud				
	Terminus of axillary bud	1st scale leaf	2nd scale leaf	Major flanking leaf	Entire axillary bud
0 Intact	0.64 ± 0.19	0.33 ± 0.24	0.20 ± 0.01	0.32 ± 0.07	1.43 ± 0.04 (0.247)[a]
0 Decapitation	0.85 ± 0.03	0.28 ± 0.04	0.33 ± 0.01	0.52 ± 0.18	1.95 ± 0.16 (0.243)
8 Intact	0.63 ± 0.16	0.23 ± 0.06	0.23 ± 0.08	0.25 ± 0.04	1.34 ± 0.06 (0.299)
8 Decapitation	1.31 ± 0.41	0.34 ± 0.02	0.35 ± 0.08	0.49 ± 0.12	2.49 ± 0.45 (0.325)
24 Intact	0.82 ± 0.02	0.36 ± 0.21	0.30 ± 0.13	0.35 ± 0.08	1.82 ± 0.41 (0.390)
24 Decapitation	2.57 ± 0.22	0.70 ± 0.29	0.79 ± 0.46	1.88 ± 0.21	5.92 ± 1.17 (0.486)

[a] Dry weight (mg/bud) of the entire axillary bud is noted in parentheses.

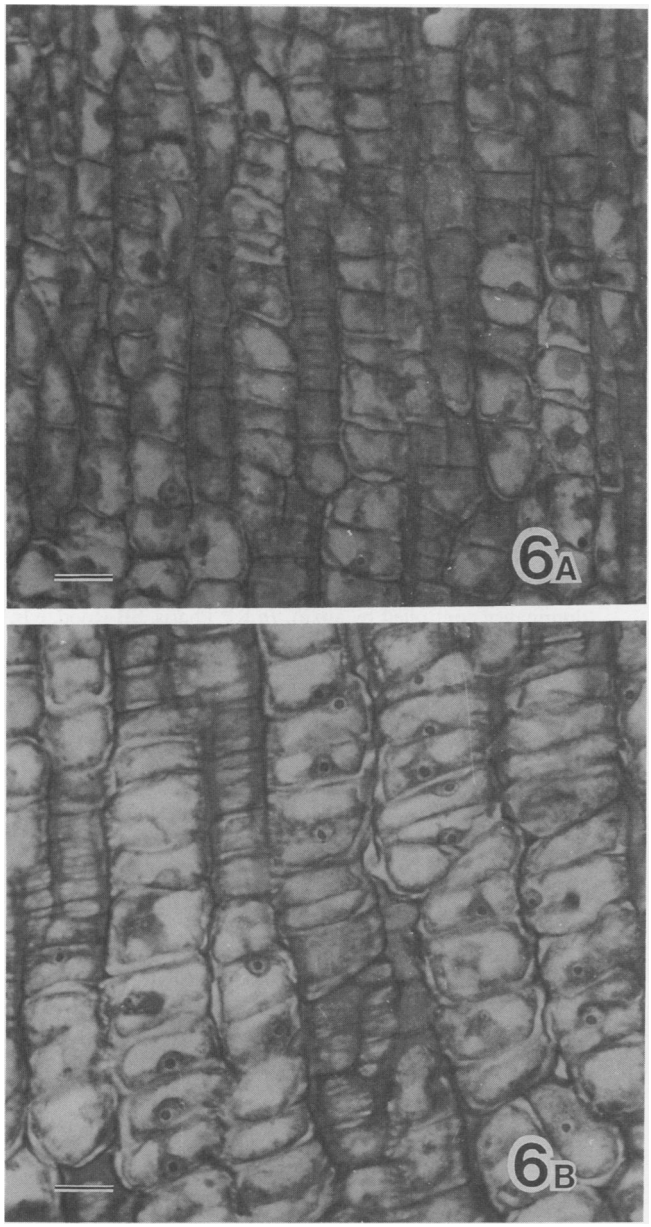

Figure 6. A, Light photomicrograph showing a longitudinal section through the internode subtending the axillary bud of an intact (control) plant sampled at the same time as the decapitated plant shown in Figure 6B. The pith parenchyma cells are square to rectangular in appearance; the long axes of the cells are parallel to the stem axis. B, Light photomicrograph showing a similar section to Figure 6A from a decapitated plant sampled 24 h after decapitation of the terminal shoot. The pith parenchyma cells have expanded in size and the long axes of the cells have become perpendicular to the stem axis. Bars = 20 μm.

As noted by Hillman (8), the IAA content of axillary buds from decapitated plants increased within 24 h after decapitation, although our IAA concentrations were fivefold higher than those found by Hillman. These differences may result from the: (a) use of a different cultivar, (b) differences in age of plants, and (c) Hillman extracted both axillary lateral buds, whereas we analyzed only the larger axillary bud.

The early increase in IAA in the larger axillary bud after decapitation may be influencing two processes: (a) its own rapid growth and (b) inhibition of other axillary buds. From the detailed time study of bud fresh weight, the increase in IAA and fresh weight concentration occur in concert. It is thus possible that increased IAA concentration may induce axillary bud growth *per se*, whereas a second messenger, possibly ethylene production triggered by IAA (15), may be required to elicit inhibition of the opposite and/or lower axillary buds. A cessation of the 'arrested state' in the larger axillary bud of decapitated plants may thus be caused by increases in IAA. The increased IAA may be prompted by the interruption of the as yet unknown 'signal' from the shoot apex (second messenger). Additionally, the cessation of the arrested state may also result, in part, from decreasing ABA concentration in the axillary lateral bud. Both Knox and Wareing (12) and Tamas *et al.* (16) observed a decrease in ABA concentration (40–60%) in the lateral bud 24 h following decapitation. The decreased concentration of ABA (30–70%) that we observed at h 24 is thus confirmatory of their results. However, further studies will be necessary to determine the exact tissue locations of the IAA increase and ABA decrease within the axillary bud.

Useful information could also be obtained from carefully timed gibberellin and cytokinin analyses, although extraction of considerably greater numbers of buds would undoubtedly be necessary to precisely and accurately measure endogenous changes in these classes of hormones.

The rhythmic changes in ABA throughout the course of 24 h (Fig. 3) are also of interest. Most of the previously reported rhythms in ABA concentration deal with stressed leaf tissue, *i.e.* peach (*Prunus persica* L. cv Fay Elberta) (18) and cotton (*Gossypium hirsutum* L.) (13). Rhythms in ABA concentration have also been noted on a daily scale, in stressed leaves of cotton (13), and on an annual scale, in lateral buds of willow (*Salix viminalis* L.) (1). Diurnal fluctuations of the order of 3 to 4 times the base value were seen in nonstressed leaf tissue of both *Arbutus unedo* (3) and peach (18). We also noted a fluctuation of 3 to 4 times the base value in axillary buds from intact plants, and 2 to 3 times the base value in buds from decapitated plants (Fig. 3). Previous published work on a daily time frame did not have sufficient data points to enable a period to be calculated. Interestingly, the period and amplitude of the flux in our study seemed to depend on whether the apical bud came from an intact or decapitated plant, decapitation causing a longer period and a smaller amplitude in ABA fluctuations.

Within 4 h following decapitation of the shoot apex, the concentration of IAA in the larger axillary bud increased a significant ($P \leq 0.007$) fivefold, and was maximal at 8 h following decapitation. In the same time frame, ABA concentration in the larger axillary bud of decapitated plants decreased by approximately half (significant at $P \leq 0.02$). Both

the increased IAA and decreased ABA concentrations were closely associated with growth changes that accompanied the release of larger axillary buds of decapitated plants from their arrested state of development. The fresh weight of the axillary bud had also increased significantly by 8 h, and anatomical analysis showed that the axillary bud had a significantly longer and wider internode segment by 8 h after decapitation of the terminal apex. This increased size was accounted for by pith parenchyma cell expansion, relative to the larger axillary bud of the intact plant.

The present work makes it clear that a more complete understanding of the mechanism of hormonal action in a specific system (such as control of apical dominance) requires (a) analysis of at least the major hormones affecting the system, and (b) time-resolved studies of changes in concentration of these hormones. Our results also identify timing of response (IAA increase, ABA decrease) to decapitation. This should help in the search for IAA- (ABA)-mediated growth mechanism(s) in control of apical dominance.

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